The Biofinder: Detection of Biological Material on Igneous Rock.

S. J. Rowley[1,2], A. K. Misra[1], T. E. Acosta-Maeda[1], and J. Zhou[3]. [1]Hawaii Institute of Geophysics and Planetology, [2]Dept. Earth Sciences, and [3]Electrical Engineering, University of Hawai'i at Mānoa, Honolulu, HI 96822, USA. srowley@hawaii.edu

Introduction:
The Biofinder is a non-invasive laser-based system, which takes advantage of the short lifetime of biofluorescent materials[1,2]. This capability enables the Biofinder to detect biological materials in real-time (1 µs) within a wide area. Notably, the Biofinder separates biofluorescence from mineral luminescence, and operates in both daytime and nighttime conditions[3]. The Biofinder instrument is capable of locating fluorescent polyaromatic hydrocarbons, amino acids, proteins, biominerals, photosynthetic pigments, microbial life and the diagenetic products of microbial life in both terrestrial and aquatic environments[1-3]. In 2018, the Biofinder was approved by NASA to a technology Readiness Level (TRL) of 4[2]. The overarching aim of this research is to detect biological materials in outer Solar System terrestrial and ocean world habitats. Therefore, to validate the Biofinder's ability to detect microbial life on igneous (basalt) rocks, results from the biofluorescence imagery were confirmed visually, using scanning electron microscopy (SEM) and quantitatively using flow cytometry.

Methods for the Detection of Biological Material on Igneous Rock: To validate the Biofinder's ability to detect microbial life on igneous rocks, small pieces of rock were aseptically sampled at the University of Hawai'i at Mānoa campus. Samples were transported in sterile 50 ml falcon tubes on ice and imaged by the Biofinder (Fig. 1a,b, 2a) prior to downstream comparative analyses. Samples were suspended in 10 ml 1xPBS pH7.3 and fixed in 0.5-2% paraformaldehyde overnight at 4ºC and treated in accordance with the analytical protocols outlined below.

Surface Microbial Communities using Flow Cytometry: To develop the Biofinders accurate quantitative detection capabilities of microbial communities, standard protocols for flow cytometry analysis were modified for igneous rock-associated microorganisms (see Byloos et al., 2018). Suspended samples were serially washed then fixed O/N as above. Serial dilutions were filtered through a 20-µm nylon mesh filter, then stained with Hoechst 34580 for 45 mins. Stained samples were analysed in a Beckman Coulter CytoFLEX S. Results reveal a dominance of hetertrophic (non-photosynthetic) bacteria (Fig. 1c), and the presence of fungi and hyphae producing a significant amount of scatter (see Fig. 2c). Additional 'noise' (left-skewed data, Fig. 2c) suggests the presence of viruses or virus-like particles (VLP's).

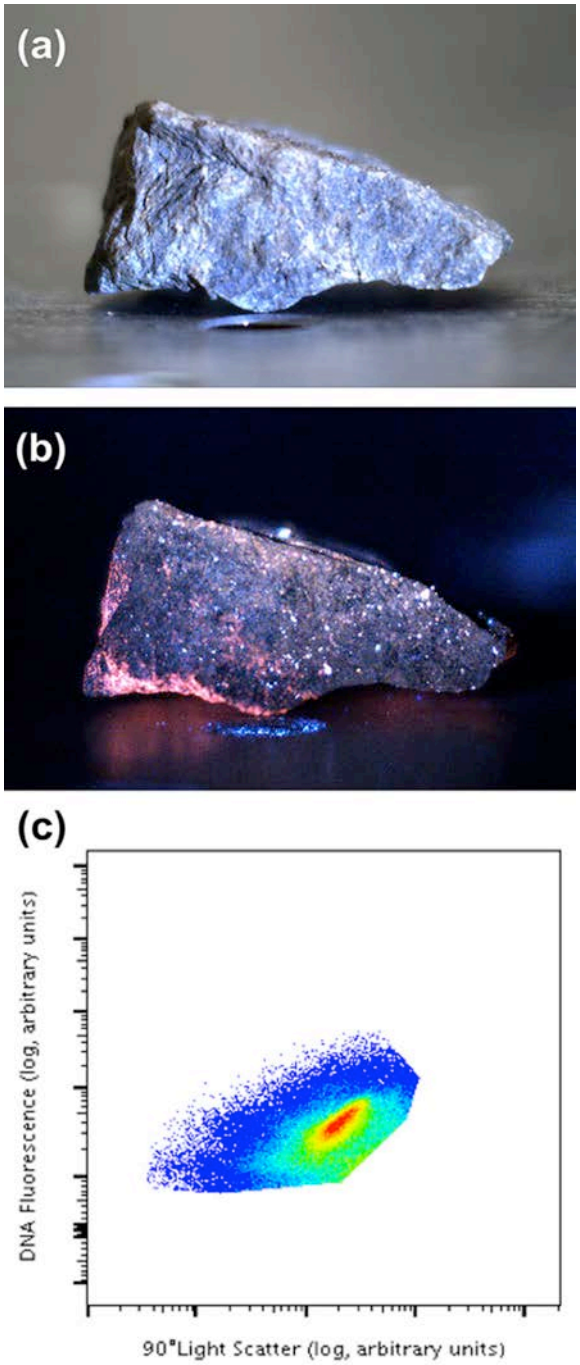

Fig. 1: Igneous rock sample images taken with the Biofinder for analysis of surface microbial communities using flow cytometry. (a) White-light image and (b) biofluorescent image of an unwashed rock sample. (c) Flow cytometry results of heterotrophic (Prokaryotes; 1.14E+0.6 cells per ml) bacteria present the rock sample.

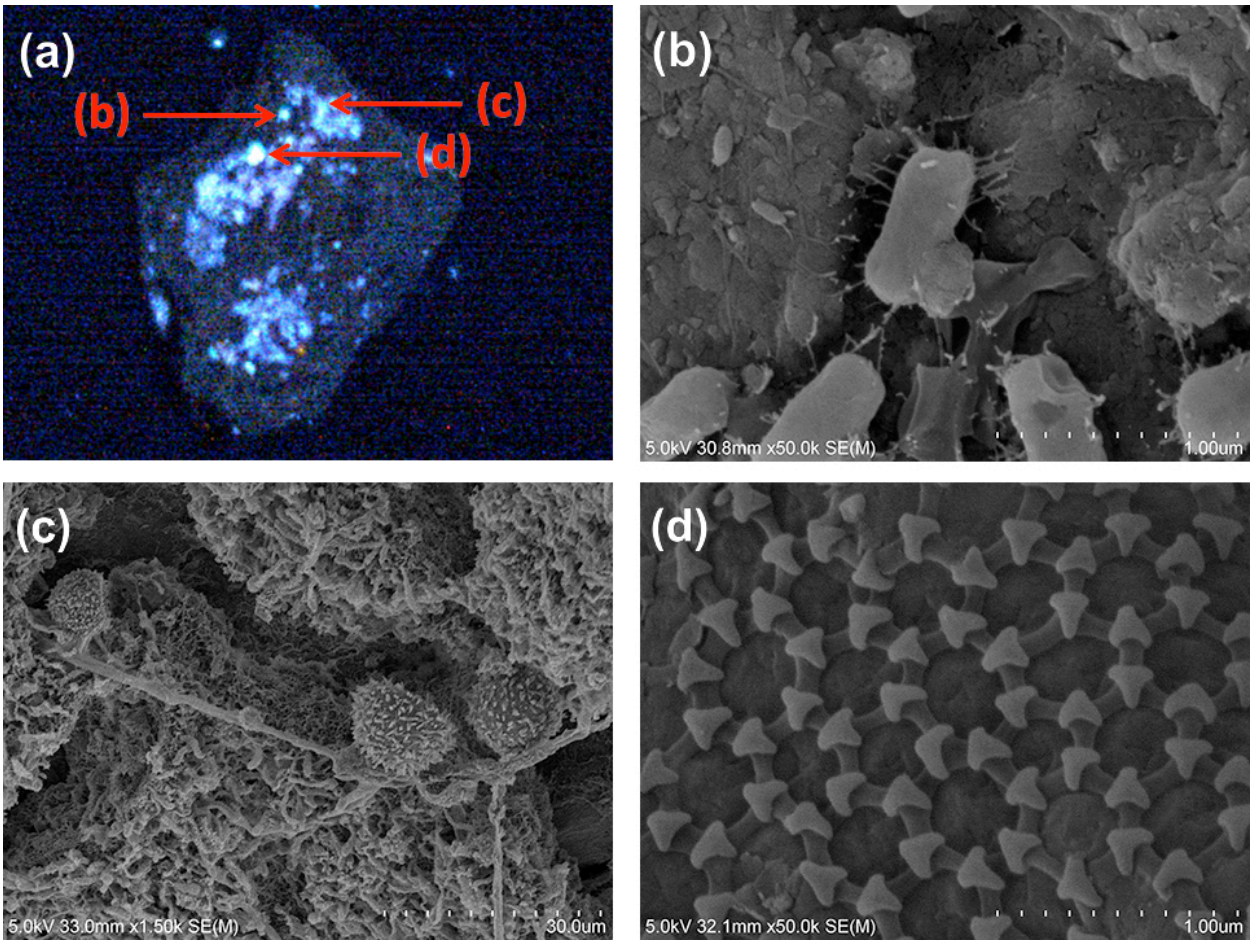

Fig. 2: Igneous rock sample image taken with the Biofinder for analysis of surface microbial communities using scanning electron microscopy (SEM). (a) Biofluorescent image of an unwashed stone sample. Red arrows depict the location of each SEM image on the unwashed stone: (b) heterotrophic bacteria (Prokaryotes), (c) fungal spores, and (d) the pheromone secretory structure of an unidentified insect.

Surface Microbial Communities using SEM: For the visualization of igneous rock-associated microorganisms from field samples, scanning electron microscopy (SEM) was used. Samples were fixed as described above, then dehydrated in a series of graded ethanol washes (200 proof, molecular grade). Samples were further dehydrated in a graded HDMS (hexamethyldisilazane): 100% EtOH and 100% HDMS series. The dehydrating procedure permits the removal of liquid in a controlled and non-destructive fashion, such that lipid membranes and cell structural integrity is maintained. The results in Fig. 2b-d confirm that the Biofinder is detecting the presence of biological matter on igneous rock field samples including prokaryotes (Fig. 2b), eukaryotes (Fig. 2c), and biogenic structures from multi-cellular organisms (Fig. 2d)

Summary: This research demonstrates the Biofinder's ability to quantitatively and qualitatively detect biological material on igneous rock that appears void of life. We will continue to develop the detection capabilities of this compact system, which could serve as a tool to detect biological carbon-based matter on 1) space instruments for planetary protection, and 2) on planetary bodies as part of a payload. Thus, facilitating the accurate yet non-invasive and non-destructive detection of astrobiological materials such as that required for future NASA Ocean World and rocky-body missions.

Acknowledgments: This work has been supported by the NASA Research Announcement (NRA), Award 16-PICASSO16_2-0032, Grant # 80NSSC17K0127. Many thanks to Dr. K. Selph at the SOEST Flow Cytometry Facility, and T. Carvahlo at the PBRC, University of Hawai'i at Mānoa.

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